Original Research Article

Antimicrobial activity of *Warburgiaugandensis* on selected standard organisms that cause urinary tract infections

Abstract

Background: The antimicrobial effect of *Warburgiaugandensis*has been recognized for many years in developing countries especially in Uganda (East Africa). However, limited investigations have ~~been~~ focused on its effect on microorganisms causing ~~Urinary~~ urinary tract infection ~~causing organisms~~.

Objective: To determine the antimicrobial activity of *Warburgiaugandensis* on selected standard microorganisms that cause urinary tract infections i.e. *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231), *Proteus mirabilis* (ATCC 25933), and *Staphylococcus aureus* (ATCC 25923).

Methods: *Warburgiaugandensis* stem bark wasobtained from Tooro Botanical centre which is located in Kabarole district a few kilometres from Fort portal town and were shade dried. The aqueous and ethanolic extracts were prepared using the decoction extraction technique and evaluation of the phytochemicals was performed using semi quantitative phytochemical screening techniques. The antimicrobial activity on three bacteria; *Staphylococcus aureus, Escherichia coli, Proteus mirabilis* and one fungus *Candida albicans* was tested by agar well diffusion and broth dilution which were used to obtain the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the plant extracts respectively.

Results: Phytochemical screeningshowed the presence of ~~Tanninstannins~~, flavonoids, alkaloids, saponins and terpenoids in both the aqueous and ethanolic extract of *Warburgiaugandensis.* All ~~the~~ testedbacteria ~~(*Staphylococcus aureus, Escherichia coli* and *Proteus mirabilis*)~~were susceptible to the aqueous extract which caused significant inhibition of microbial ~~growth.~~ growth with the highest activity observed on *Staphylococcus aureus* which had an MIC of 0.49mg/ml,~~While~~ whileall the bacteria were less susceptible to the ethanolic extract when compared to the standard susceptibility ranges of ciprofloxacin.~~The standard organisms were more susceptible to the aqueous extract with the highest activity~~

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~~observed on *Staphylococcus aureus* (ATCC 25923) which had an MIC of 0.49mg/ml.~~ The same trend was observed in the ethano~~l~~lic extract where *Staphylococcus aureus* (ATCC 25923) had an MIC of 1.95mg/ml compared to *Escherichia coli* (ATCC 25922) which had 62.5mg/ml. A similar trend was observed also with the MBC/MFC. *Candida albicans* also showed more susceptibility on aqueous extract than the ethanolic.

Conclusion: The aqueous and ethanolic extracts of *Warburgiaugandensis*exhibit antimicrobial effect against the selected urinary tract infection causing organisms. The aqueous extract shows antimicrobial activity in both agar well diffusion and broth dilution method. This study further shows the potential of *Warburgiaugandensis*being a novel source of modern drugs with further studies and these results provide some new perspectives on the traditional uses of *Warburgiaugandensis* in treating urinary tract infections.

Key words: *Warburgiaugandensis*, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, Minimum Fungicidal Concentration, ~~antimicrobial~~ Antimicrobial activity. *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Candida albicans*.

Introduction

Warburgiaugandensis Sprague (Family Canellaceae), commonly known as "Ugandan Greenheart ~~Heart Tree~~tree", is an evergreen plant, which is mainly distributed in Eastern and Southern Africa (references). For generations, traditional healers ~~were~~ have beenusing *Warburgiaugandensis* extracts made of bark, roots or leaves to treat different kinds of diseases/ailments like malaria, tuberculosis, skin diseases, ulcers, lung problems or intestinal worms, to name a few (references). *Warburgiaugandensis* more pharmacognosy description family is also known as **Ugandan greenheart**, and is a species of evergreen tree native to Africa. The plant is found in the following countries; Kenya, Uganda, Ethiopia and some parts of western Africa (D. Olila, Olwa, & Opuda-Asibo, 2001). The wood of this plant is used for timber, firewood, poles, charcoal, stools, carvings, spoons, treatment of many human diseases including stomach-ache, fever, colds, headache, stomach-ache, hernia, malaria, toothache, intestinal problems, generalized body pains, fatigue, constipation (Lovett, Ruffo, Gereau, & Taplin, 2006; Van Wyk, 2008).

Urinary tract infections (UTIs) are amongst the most common human infections globally. Indeed, it has been estimated that nearly 800 million people (equating to approximately 11% of the global population) develop at least one UTI in any given year [1, 2]. They are substantially more common in women than in men, with the prevalence in women estimated

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to be approximately five times higher than in males [3]. Indeed, it is expected that more than half of female population of the world will contract at least one UTI in their lifetime, with a substantial proportion experiencing recurrent infections (reference).

Urinary tract infections are classified as either complicated or uncomplicated. Complicated infections occur in people with underlying conditions or abnormalities in any part of the genitourinary tract, making the infection more serious and more challenging to treat than uncomplicated infections. In contrast, uncomplicated UTIs are classified as infections occurring in the absence of comorbidities or other anatomical urinary tract and renal abnormalities (references).

Interestingly, there can be notable differences between the infectious agents responsible for uncomplicated and complicated UTIs. The vast majority of these pathogens are normal ~~components~~ flora of the gastrointestinal or vaginal microflora, thereby increasing the chances that they cause UTIs. For both classes of UTI, uro-pathogenic *Escherichia coli* are the leading infective agent, accounting for approximately 75 and 65% for uncomplicated and complicated UTIs, respectively (references).

Notably, complicated UTI-causative pathogens are linked to increased rates of antimicrobial resistance (references). Therefore, the development of effective therapies to treat these conditions is vital, not only to decrease the effects of these infections, but also to slow the development of further antibiotic-resistant bacterial strains.

In Uganda, ~~Antimicrobial~~ antimicrobial resistance (AMR) is a threat that needs to be addressed to minimize its associated negative effects. It is estimated that AMR could lead to 10 million deaths globally per year by 2050 and a USD $100 trillion economic loss if no action is taken(Bassetti et al., 2017). There is need to supplement the current antimicrobial treatment regimen to minimize development of ~~Antimicrobial~~ antimicrobial resistance (references). The medicinal components in *Warburgiaugandensis* plant could be a solution (Dilbato, Begna, & Joshi, 2019). The plant is readily available and accessible to the indigenous communities;however, little is known about its activity on common UTI causing pathogens. The understanding of antimicrobial activity of *Warburgiaugandensis* on these prevailing urinary tract pathogens could become an alternative herbal treatment option to supplement on the current antimicrobial treatments.

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MATERIALS AND METHODS

Study Design

This study was ~~Laboratory~~ laboratory based in which the actives of *Warbugiaugandensis* were extracted by boiling in hot water and the present phytochemicals determined. The *Warbugiaugandensis* aqueous and ethanolic extracts were used to determine the antimicrobial activity against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933), and *Candida albicans* (ATCC 10231).

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Study area

Warburgiaugandensis plant was obtained from Tooro Botanical centre which is located in Kabarole district approximately 1.5 kilometres from Fort portaltown. ~~A variety of plant species especially medicinal plants are grown there. However, it can be found in other areas in Uganda which include the districts of Kabale, Mpigi, Iganga, and Moroto (Gumisiriza, Birungi, Olet, & Sesaazi, 2019).~~ The standard organisms ~~we~~ used were obtained from Microbiology Laboratory of Mbarara University of Science and Technology.

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The study was carried out at Mbarara University of Science and Technology where the extraction process was done from the Pharmaceutical Analysis/Chemistry Laboratory of Mbarara University of Science and Technology. The antimicrobial studies were carried out in the Microbiology laboratory of Mbarara University of Science and Technology. Microbiology laboratory of Mbarara University serves a population of over four million people in its catchment area comprising the districts of Mbarara, Bushenyi, Ntungamo, Kiruhura, Ibanda, Buhweju, Rubirizi, Mitooma and Isingiro. The hospital also receives patients from Kabale, Masaka, Fort Portal and neighbouring countries like Rwanda and Tanzania. In south- western Uganda, majority of the population are living below the poverty line thus *Warburgiaugandensis* plant is a good treatment option for them if proven effective.

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~~**Plant material**~~

~~*Warburgia ugandensis* plant extracts have been reported to show some antimalarial, antifungal, and antimicrobial properties. The stem bark was used in this study because it accumulates more phytochemical compared to other parts of the tree (Okello, Komakech, Matsabisa, & Kang, 2018). The bark of the plant was obtained from Tooro Botanical centre~~

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~~which grows different species of plants and local herbs for research and commercial purposes.~~

Preparation of the extract

The stem barks which were harvested bydebarkingthe tree using a sharp-edged~~machete~~machete were cleaned using a hard brush to remove dirt and soil particles. They were then chopped into small pieces which were then air dried ~~away from direct sunlight~~ for 3 weeks until they were completely dry.

Aqueous Extraction (Decoction): The dried stem barks were ground using a motor and a pestle and sieved using 250nm sieve to obtain a fine powder. 500g of the dried stem bark powder was then weighed and added to 2600ml of boiling distilled water and the mixture boiled for 50 minutes. The decoction was cooled and then filtered with a ~~mucilin~~muslin cloth and then with cotton wool using a Buchner funnel. The filtrate was then concentrated using a rotary evaporator (Brand, Manufacturer)and the resultant concentrate was freeze dried (Brand, Manufacturer) to obtain a powder. The dried extract was further crushed and then sieved using a 700nm sieve and later a 250nm sieve so as to obtain a finer powder. The aqueous extract obtained weighed 34.7g.

Ethanolic Extraction (Maceration): The dried stem barks were ground using a motor and a pestle and sieved using 250nm sieve to obtain a fine powder. 500g of the dried stem bark powder was then weighed and added to 2000 ml of absolute ethanol in a glass maceration container.The container was closed and agitation was done once per day for 7 days. After the 7 days of extraction, the extract was then filtered using a ~~mucilin~~muslin cloth and then with cotton wool using a Buchner funnel. The filtrate was concentrated using a rotary evaporator (Brand, Manufacturer)with a revolution of 95 revolutions per minute for 3hoursat 50^0C. A paste like substance was then obtained and it weighed 119.3g. The resultant concentrate was freeze driedto obtain a powder. The dried extract was further crushed and then sieved using a 700nm sieve and later a 250nm sieve so as to obtain a finer powder. The aqueous extract obtained weighed 38.6g.

Phytochemical ~~screening~~Screening

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Two grams of powder eachof the ~~Ethanolic~~ ethanolic and aqueous extract of *Warburgiaugandensis*stem bark ~~were~~ wasdissolved in 20ml~~s~~ of absolute ethanol and 20 ml~~s~~ of distilled water respectively to form stock solutions which were used for phytochemical screening.

The preliminary phytochemical analysis of the prepared plant extracts ~~were~~ was carried out using standard methods that is; Tannins [Ferric Chloride test], Amino acids [Ninhydrin test], alkaloids [Drangendorffs test], saponins [Frothing test], terpenoids [Chloroform and concentrated H_2SO_4], glycosides [Benedict's test], flavonoids [Ammonia test] and reducing sugars [Benedict's test].

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Microbiological Assay

The organisms used were standard strains of *Escherichia coli* (ATCC 25922),*Proteus mirabilis* (ATCC 25933),*Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) which were obtained from MUST Microbiology laboratory. The agar well diffusion method was used to determine the Minimum Inhibitory Concentration(MIC) for both the Ethanolic and aqueous extracts of *Warburgiaugandensis* against the standard strains. The broth dilution method was used to determine the Bactericidal Concentration(MBC)and the Fungicidal activity (MFC) for both the Ethanolic and aqueous extracts of *Warburgiaugandensis* against the standard bacterial and fungal organisms respectively.

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Preparation of bacterial culture suspension: Upon sub-culturing the reference strains on Nutrient agar, a colony of each organism was emulsified in 1.5 mL of distilled water. The density of the bacteria culture suspension to be used for the tests was adjusted for the McFarland standard 0.5 (1.5×10^8 Colony Forming Units/ml).

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Determination of MIC by agar well diffusion: Serial dilutions were made to be dispensed on culture plates inoculated with the standard organisms. The Minimum Inhibitory Concentration (MIC) was established by measuring the zone of clearance observed on the culture plate after subjecting standard organisms to the different dilutions of the extracts in agar wells. The lowest concentration of the extracts for which clearance was observed was then taken as the MIC. For both the aqueous and ethanolic extracts, the zone of clearance at

different dilutions of the extract reflected organism's susceptibility and resistance patterns to the *Warburgiaugandensis* extract. *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Candida albicans* had different MICs as shown in the table 4 of the results.

Preparation of working solutions: Serial dilutions of aqueous and ethanolic extracts of *Warburgiaugandensis*were made using Nutrient Broth using a stock solution of 500mg/ml concentration to obtain 11 fold dilutions (~~neat, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048 giving concentrations of~~ 250mg/mL, 125mg/mL, 62.5mg/mL, 31.25mg/mL, 15.63 mg/mL, 7.81mg/mL, 3.91mg/mL, 1.95mg/mL,0.98mg/mL, 0.49mg/mL, and 0.24mg/mL respectively(Mwitari, Ayeka, Ondicho, Matu, & Bii, 2013).

Preparation of agar well diffusion plates: Two sterile plates of MHA were used in each test. One of these plates was inoculated with the test organism; the other was left ~~not~~ uninoculated and served as a check for media sterility (Mekonnen, 2010). Using a sterile cotton swab, the inoculum onto the Mueller Hinton Agar was made to form a microbial lawn. Using the bottom of pipette tips, wells were dug, 8 mm diameter into the Mueller Hinton Agar (5 wells for each plate). 100µl volume of the different *Warburgiaugandensis* extract dilutions were dispensed into the wells including the positive control which was a known antibiotic Ciprofloxacin(≤0.25mcg/ml), known antifungal Fluconazole (0.125-64µg/ml) and phosphate buffered saline as the negative ~~Control~~ control (Khumalo, Sadgrove, Van Vuuren, & Van Wyk, 2019). The extract was allowed to diffuse and there after ~~we~~ wasincubated in an upright position at 37^0c for 24 hours. After 24hrs, the plates were read for zones of inhibition and measured using a millimetre ruler. The plates were inverted and incubated for more 24 hours and another reading of zone diameter was recorded. The procedure was repeated 3 times on the same organism using the same extracts to get the average.

Determination of Minimum Bactericidal Concentration (MBC) by broth Dilution method: Serial dilutions of aqueous and ~~Ethanolic~~ ethanolic extracts of *Warburgiaugandensis*were made using Nutrient Broth using a stock solution of 500mg/ml concentration to obtain 11 fold dilutions (~~neat, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048 giving concentrations of~~ 250mg/mL, 125mg/mL, 62.5mg/mL, 31.25mg/mL, 15.63 mg/mL, 7.81mg/mL, 3.91mg/mL, 1.95mg/mL,0.98mg/mL, 0.49mg/mL, and 0.24mg/mL respectively). 200µl of the different standard microbial and fungal suspensions were dispensed into each tube and incubated for 48hours at 37^0C. Subcultures of the suspensions from the respective bottles were made on Mueller Hinton Agar and chocolate

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Agar plates incubated at 37^0C for 24hrs hours. Plates were read and the highest dilution which gave no growth on the Agar plates was recorded as the MBC

Quality ~~control~~Control~~.~~

The appropriate temperatures and revolutions (for the rotary evaporator) for the extraction procedures were 50^0C and 95 revolutions per minute for the ~~Ethanolic~~ ethanolic extract respectively. The stem bark extracts were also stored in dark amber bottles to prevent deterioration caused by ultra violet light on the active substances.

The culture plates were stored at 2-8^0C and autoclaved for sterility at 121^0c for 15 minutes, the colour and ~~PH~~ pHof the media was checked and each new batch of agar was tested with control strains for example *Enterococcus faecalis* (ATCC 29212 or 33186). ~~Ciprofloxacin (≤0.25mcg/ml) and Fluconazole (0.125-64µg/ml) were used as positive controls for the bacterial and fungal test organisms, respectively while phosphate buffered saline was used as the negative control.~~

Data ~~analysis~~Analysis

The data collected which included; zone diameter of inhibition, MIC and MBC of both the ~~Ethanolic~~ ethanolic and water extract of *Warburgiaugandensis* was entered into Microsoft Excel 13, which was used to obtain the mean diameter for the zones of inhibition.

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Results

Phytochemistry findings

The phytochemical screening of the *Warburgiaugandensis*aqueous and ~~Ethanolic~~ ethanolic extract found a positive reaction to tannins, flavonoids, alkaloids, saponins and terpenoids which were all abundant in the aqueous extract while the ~~Ethanolic~~ ethanolic extract had an abundance of flavonoids and alkaloids with moderate tannins and terpenoids and less pronounced saponinsand no detectable reducing sugars (table 1).

Table 1. Phytochemical constituents of *Warburgiaugandensis* aqueous and ~~Ethanolic~~ ethanolic extracts

Constituents	Aqueous extract	Ethanolic extract
Tannins	+++	++
Flavonoids	+++	+++
Alkaloids	+++	+++
Saponins	+++	+
Terpenoids	+++	++
Reducing sugars	+++	Absent

Key: High (+++), Moderate (++), Low (+).

Antimicrobial Activity

Both the aqueous and ~~Ethanolic~~ ethanolic extract demonstrated antimicrobial activity. However, it was more pronounced in the aqueous extract which showed larger zones of inhibition as shown in table 2 and table 3. The aqueous extract showed more antimicrobial activity against *Staphylococcus aureus* with a zone inhibition diameter of 30mm followed by *Proteus mirabilis, Escherichia coli and Candida albicans* with 28mm, 27mm and 27mm diameter respectively as shown in table 2.

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Table 2. ~~Zone~~ Diameter of Zone of inhibition (mm) due to activity of aqueous *Warburgiaugandensis*plant extract

	Negative control(mm)	Diameterof Zone ~~diameter~~ of inhibition due to activity of Aqueous extract(mm)	Positive control(mm)
Staphylococcus aureus	0	30	29
Escherichiacoli	0	27	32
Proteus mirabilis	0	28	38
Candida albicans	0	27	28

Table 3. ~~Zone~~ Diameter of Zoneof inhibition (mm) due to activity of ethanolic*Warburgiaugandensis*plant extract

	Negative control(mm)	Zone diameter of inhibition due to activity of Ethanolic extract	Positive control(mm)
Staphylococcus aureus	0	20	28
Escherichia coli	0	15	35
Proteus ~~species~~mirabilis	0	18	35
Candida albicans	0	15	33

Note: Susceptibility ~~range for ciprofloxacin are~~ranges for ciprofloxacin are

Sensitive >= 31mm, Intermediate (21-30) mm, Resistant <= 20mm

Minimum Inhibitory Concentration

Both the aqueous and ~~Ethanolic~~ ethanolic extract showed low ~~concentrations of~~ MIC. However, the ~~Aqueous~~ aqueous extract had lower MIC values for example, the MIC for the aqueous extract against *Proteus mirabilis* was the lowest at 0.24 mg/ml followed by 0.49mg/ml against *Staphylococcus aureus* whereas the highest MIC of 0.98 mg/ml for the aqueous extract was against *Escherichia coli* and *Candida albicans*. The ~~Ethanolic~~ ethanolic extract however showed higher MIC values the highest being 62.5 mg/ml against *Escherichia coli* followed by an MIC of 31.25 mg/ml against *Proteus mirabilis* while the lowest MIC

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values (more antimicrobial activity) were 15.63 mg/ml and 1.95mg/ml against *Candida albicans* and *Staphylococcus aureus* respectively.

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Table 4. Minimum Inhibitory Concentration (mg/ml) of *Warburgiaugandensis*plant against standard organisms.

	***Staphylococcus aureus* (ATCC25923)**	***Escherichia coli* (ATCC25922)~~,~~**	***Proteus mirabilis* (ATCC25933)~~,~~**	***Candida albicans* (ATCC10231)~~,~~**
Aqueous extract	0.49	0.98	0.24	0.98
Ethanolic extract	1.95	62.5	31.25	15.63

Note: Standard MIC range for; Ciprofloxacin (positive control) is 0.12 µg/mL to 1 µg/mL, Fluconazole (positive control) is 0.03mg/ml to 16mg/ml

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Minimum Bactericidal Concentration

Both the aqueous and ~~Ethanolic~~ ethanolic extract showed low concentration of MBC. However, the ~~Aqueous~~ aqueous extract had lower MBC values for example, ~~The~~ the MBC of the *Warburgiaugandensis* aqueous extract was 7.81mg/ml, 62.5mg/ml, 125mg/ml, 15.63mg/ml against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933) and *Candida albicans* (ATCC 10231) respectively; While the MBC of the *Warburgiaugandensis* ethanolic extract was 7.81mg/ml, 125mg/ml, 250mg/ml, 62.5mg/ml against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933) and *Candida albicans* (ATCC 10231) respectively.

Table 5*:* Minimum Bactericidal Concentration (MBC)(mg/ml) of *Warburgiaugandensis*plant extract

	***Staphylococcus aureus* (ATCC25923)**	***Escherichia coli* (ATCC2592<u>2</u>)**	***Proteus mirabilis* (ATCC2593<u>3</u>)**	***Candida albicans* (ATCC10231)**
Aqueous extract	7.81	62.5	125	15.63
Ethanolic extract	7.81	125	250	62.5

Discussion

The phytochemical screening of *Warburgiaugandensis*ethanolic and aqueous extracts showed the presence of tannins, flavonoids, alkaloids, saponins and terpenoids which match those reported by Denis Okello and his colleagues in 2018. Concentration of tannins, flavonoids, alkaloids, and terpenoids in the *Warburgiaugandensis*extracts was high which are believed to be responsible for the antimicrobial activity(Okello et al., 2018).

As the need for the use of medicinal herbs especially, in the rural communities increases, there is a great need for studies that will assist in safe and effective use of herbal formulations (Adiukwu, Amon, & Nambatya, 2011). In this study, the antimicrobial activity of both aqueous and ethanolic *Warburgiaugandensis* extracts on four standard organisms was determined. ~~The MIC was determined using the agar well diffusion method and the MBC/MFC was determined using the broth dilution method.~~

The results of this study indicated that both aqueous and ethanolic extracts of *Warburgiaugandensis* had an antimicrobial activity on the test organisms but with higher susceptibility of organisms to the aqueous extract than the ethanolic extract as shown by the MIC and MBC/MFC results in table 4 and table 5. This is in agreement with a study carried out by D Olila that also concluded that aqueous *Warburgiaugandensis* extracts were more effective than the ethanolic extracts on the test organisms(D Olila & Opuda-Asibo, 2001) unlike the study done by Njire, Bundabula and Kiiru which showed that alcoholic extracts to have more activity than the aqueous extracts (Njire, Budambula, & Kiiru, 2021). The aqueous extract of *Warburgiaugandensis* showed remarkable activity against all the test organisms with the highest activity at a MIC value of 0.24 mg/ml against *Proteus mirabilis* and MIC of 0.49 mg/ml against *Staphylococcus aureus*. The aqueous extract had the least activity against *Escherichia coli* and *Candida albicans* with MIC value of 0.98 mg/ml which agrees with the study done by YibeltalmerawieBetsehaon the antimicrobial activity of crude and semi-purified fractions of *Warburgiaugandensis* against some pathogens. The ethanolic extract of *Warburgiaugandensis*followed a similar trend and showed the highest activity on *Staphylococcus aureus* with MIC of 1.96 mg/ml and the least activity on *Escherichia coli*, *Proteus mirabilis* sand *Candida albicans* with MIC of (62.5, 31.25 and 15.63) mg/ml respectively (Merawie, Sahile, Moges, & Husen, 2013).

Ethanol extract of *Warburgiaugandensis*had a lower MIC and MBC/MFC in comparison to the aqueous extract against tested microbial strains. The difference could be attributed to the

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Comment [cyp65]: This is a repetition of results, authors should avoid repeating results in this section but focus on discussing the results only

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fact that during phytochemical analysis there was variation in the phytochemical component concentration. The aqueous extract contained a higher concentration of tannins, flavonoids and terpenoids which have been associated with the antimicrobial activity of this plant (Okello & Kang, 2019).

Conclusion: The aqueous and ~~Ethanolic~~ ethanolic extracts of *Warburgiaugandensis*have considerable effect on the pathogenscausing~~Urinary~~ urinary tract ~~infection~~ infection~~causing pathogens~~. The aqueous extract has better antimicrobial activity in both agar well diffusion and broth dilution method. This study shows the potential of *Warburgiaugandensis*which may be adopted for treatment of urinary tract infections.The results from this study will also provide some new perspectives on the traditional uses of *Warburgiaugandensis* in treating urinary tract infections.

Acknowledgement?

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Comment [cyp67]: Follow format

Comment [cyp68]: I disagree with authors. The MIC values obtained are big thus showing a weak antimicrobial activity for a crude extract, so extract cannot be used for UTI management. Secondly, the toxicity of the extract has not been established by the authors on this manuscript, they cannot suggest it to be used for management of UTIs, Third, this was an invitro lab based study, it cannot be used to confidently suggest use unless followed by proper studies invivo

Comment [cyp69]: Authors did not follow journal format for reference writing

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